

## HSV-creGFP

## HSV-OVA

phase contrast fluorescence

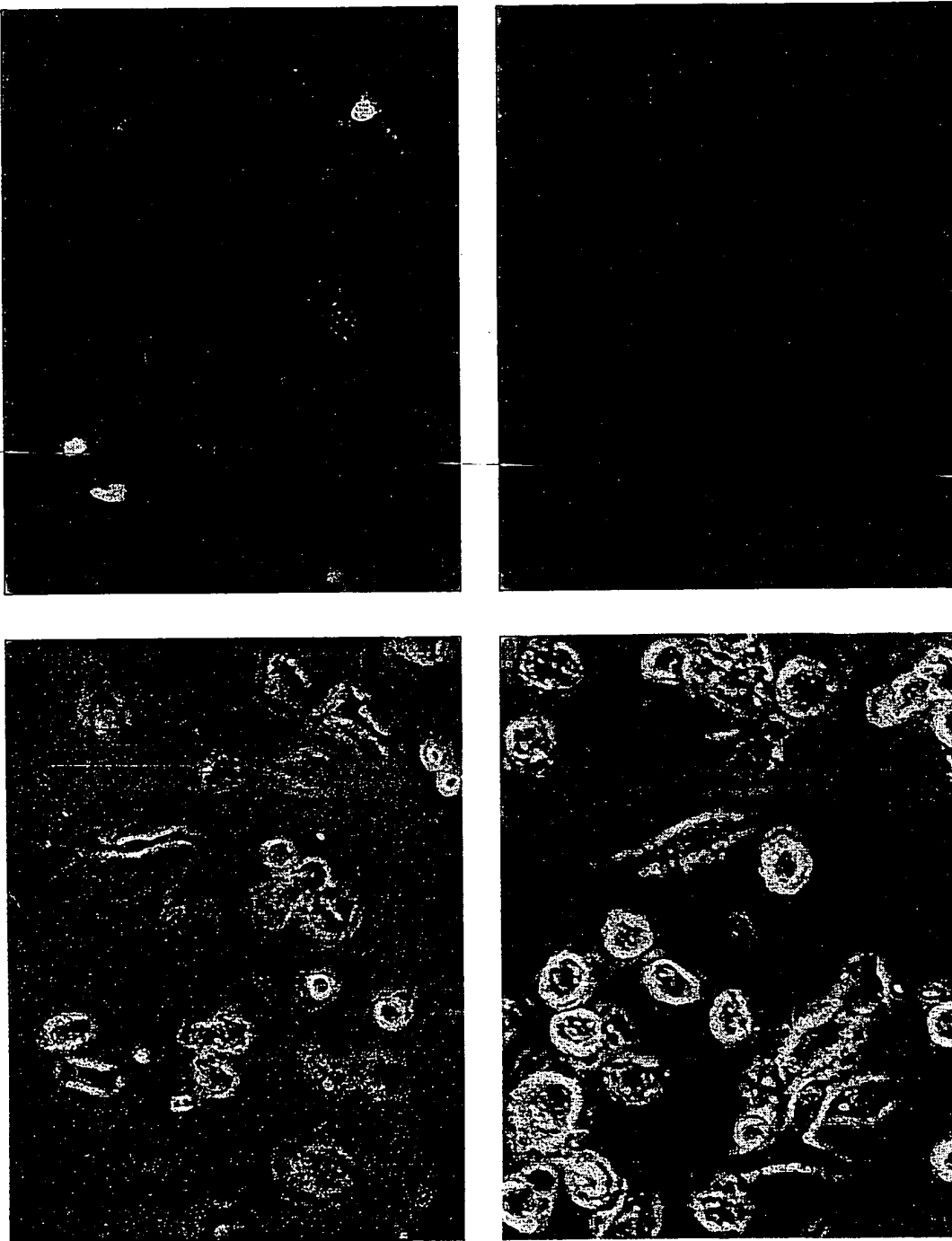
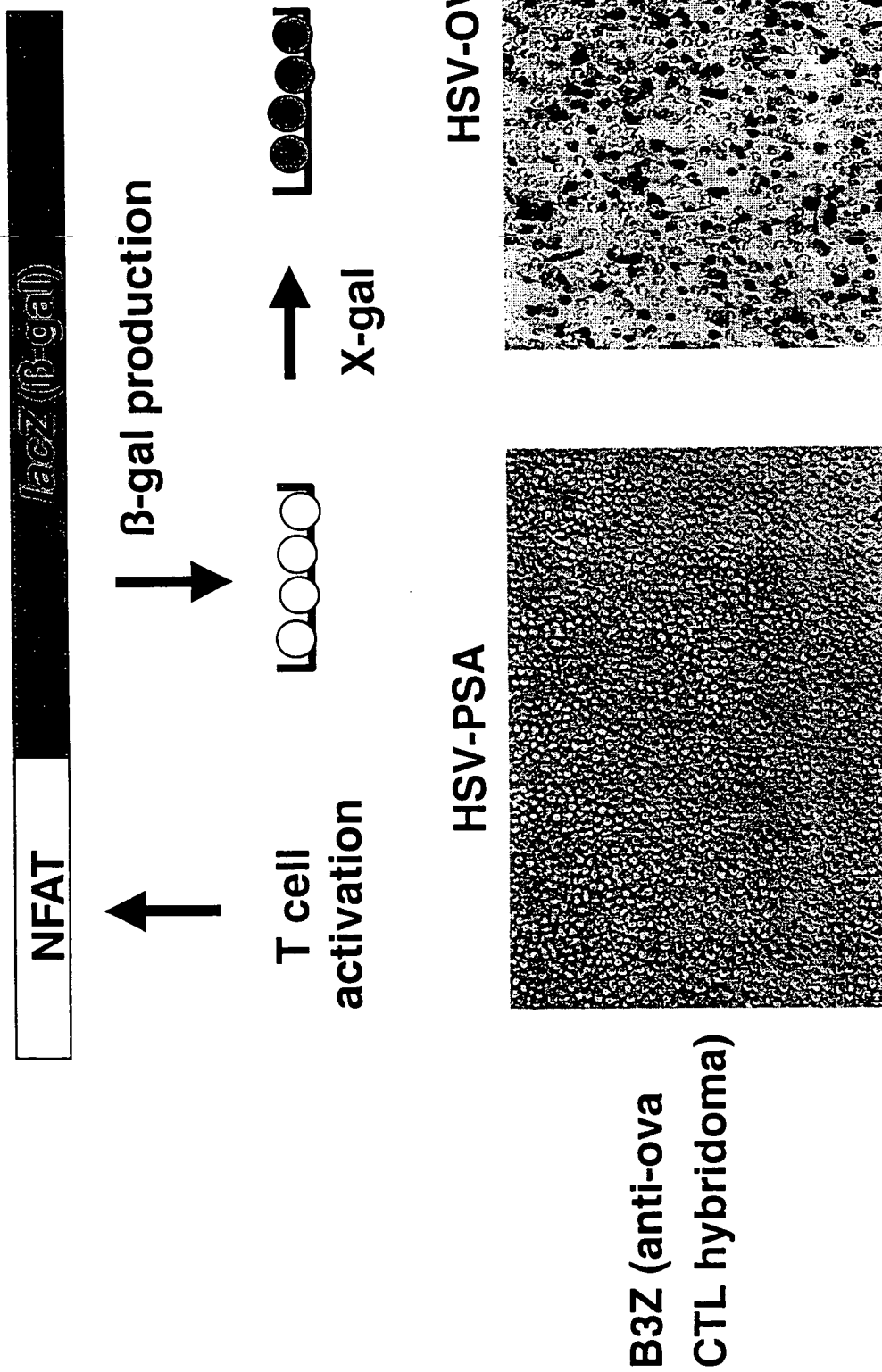
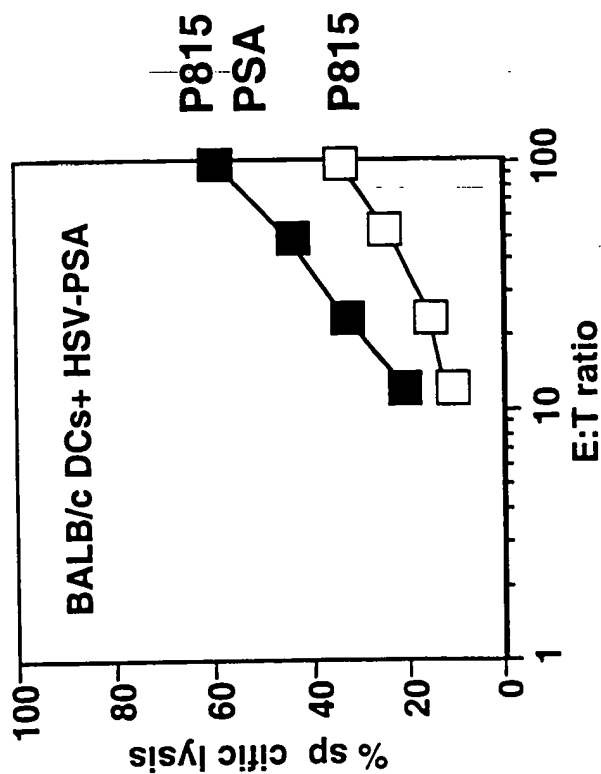
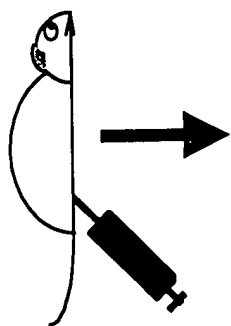


Figure 1: HSV amplicon vector-mediated transduction of murine dendritic cells. Dendritic cells were infected overnight with HSV-creGFP or HSV-OVA amplicons (MOI=1) as a negative control and were directly visualized by fluorescence microscopy without fixation.

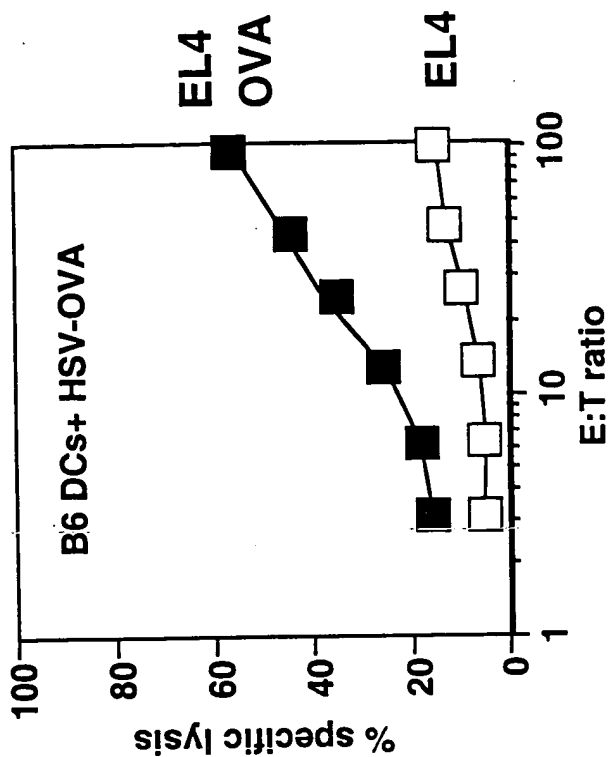
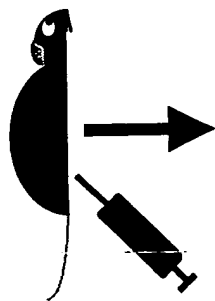


**Figure 2: Dendritic cells infected with HSV amplicons present antigen to T cell hybridomas.** DCs from a (C57BL/6 x BALB/cByJ)F1 mouse were infected with HSV-OVA and cultured overnight with CTL hybridoma B3Z (specific for OVA). These hybridomas have been previously transfected with *lacZ* under control of the IL-2 promoter and can be assayed for activation by staining with X-gal. Blue cells represent activated hybridomas and indicate that the DCs have been transduced and are capable of processing the OVA for class I MHC presentation.

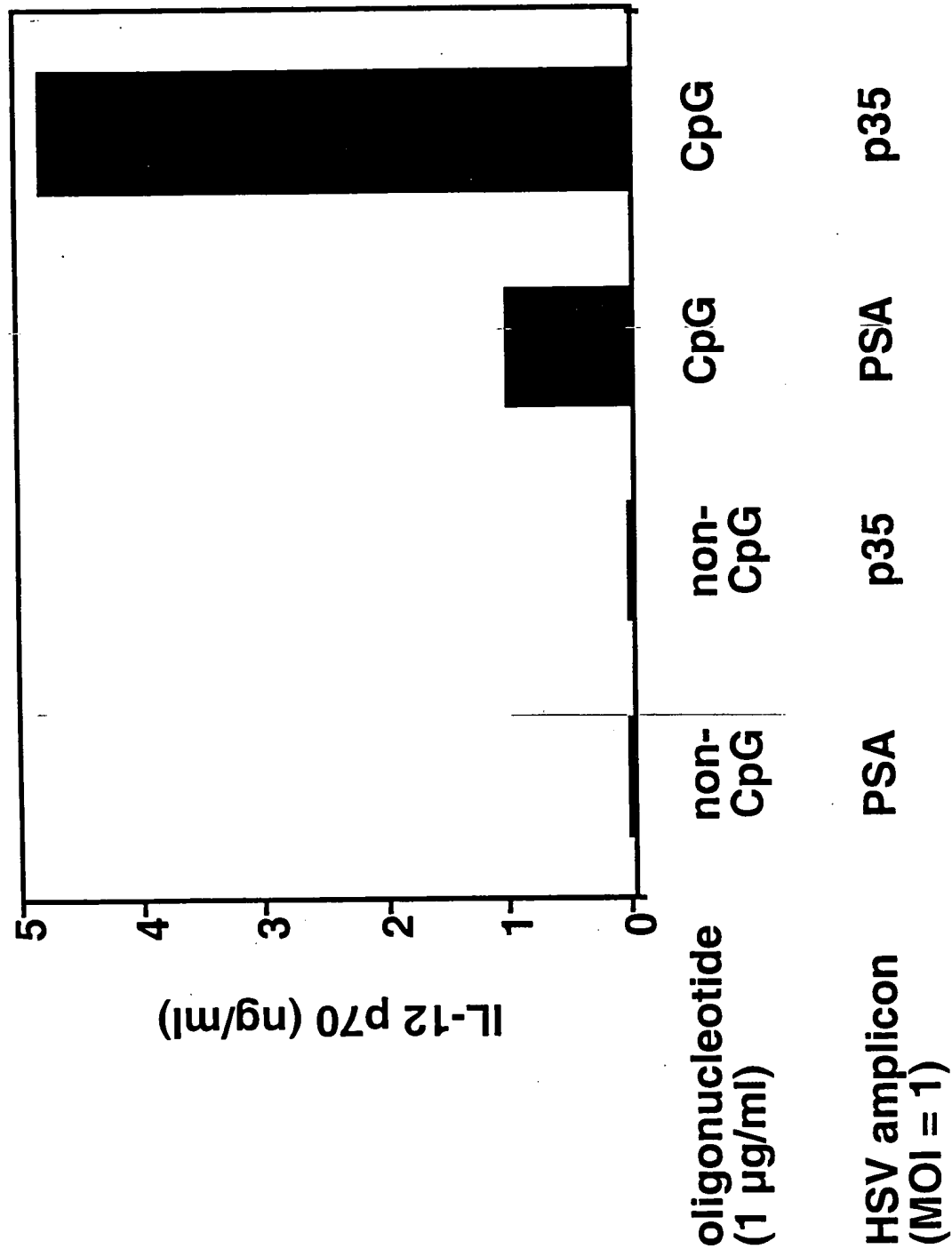
# **BALB/c immunized twice with DCs+HSV-PSA**



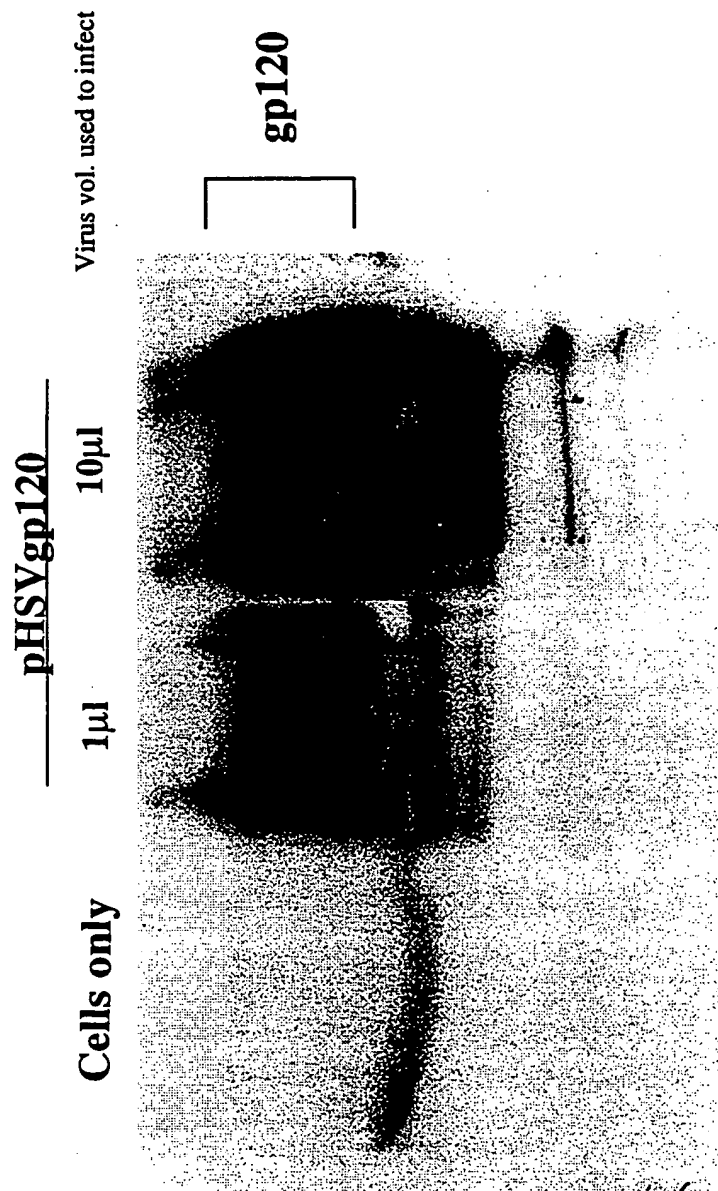
# **C57BL/6 immunized twice with DCs+HSV-OVA**



**Figure 3: Mice immunized with HSV amplicon-transduced dendritic cells elicit specific cytotoxic T cell responses.** Dendritic cells were infected with amplicons at an MOI of 1 and transduced cells were used to immunize mice twice subcutaneously 1 week apart. Splenocytes from immunized animals were re-stimulated *in vitro* for 5 days with irradiated, lipopolysaccharide-treated B cell blasts pulsed with the immunodominant peptide of PSA or OVA. CTL responses were measured using a standard <sup>51</sup>Cr release assay. "E:T ratio" refers to the effector cell to target cell ratio.



**Figure 4: Dendritic cells infected with HSV-p35 amplicons and activated with CpG oligos produce increased levels of IL-12 p70 heterodimer.** DCs were infected with HSV amplicons engineered to express the p35 subunit of IL-12, or HSV-OVA amplicons as a control. Cells were then activated overnight with oligonucleotides that contain an immunostimulatory CpG sequence or control oligos in which the CpG sequence is altered to GpC. Supernatants were collected 48 hours later and tested in an IL-12 ELISA specific for IL-12 p70 heterodimer.



**Figure 5: Western blot analysis of lysates prepared from HSV gp120-infected NIH 3T3 cells.** A 20-μg sample of cell lysate isolated from uninfected and HSV gp120-infected NIH 3T3 cells were electrophoretically separated on a 10% SDS-PAGE gel, transferred to nylon membrane, and blot incubated with a HIV gp120-specific antibody (Clontech, Inc.). The gp120-specific bands were visualized on film using chemiluminescent detection.

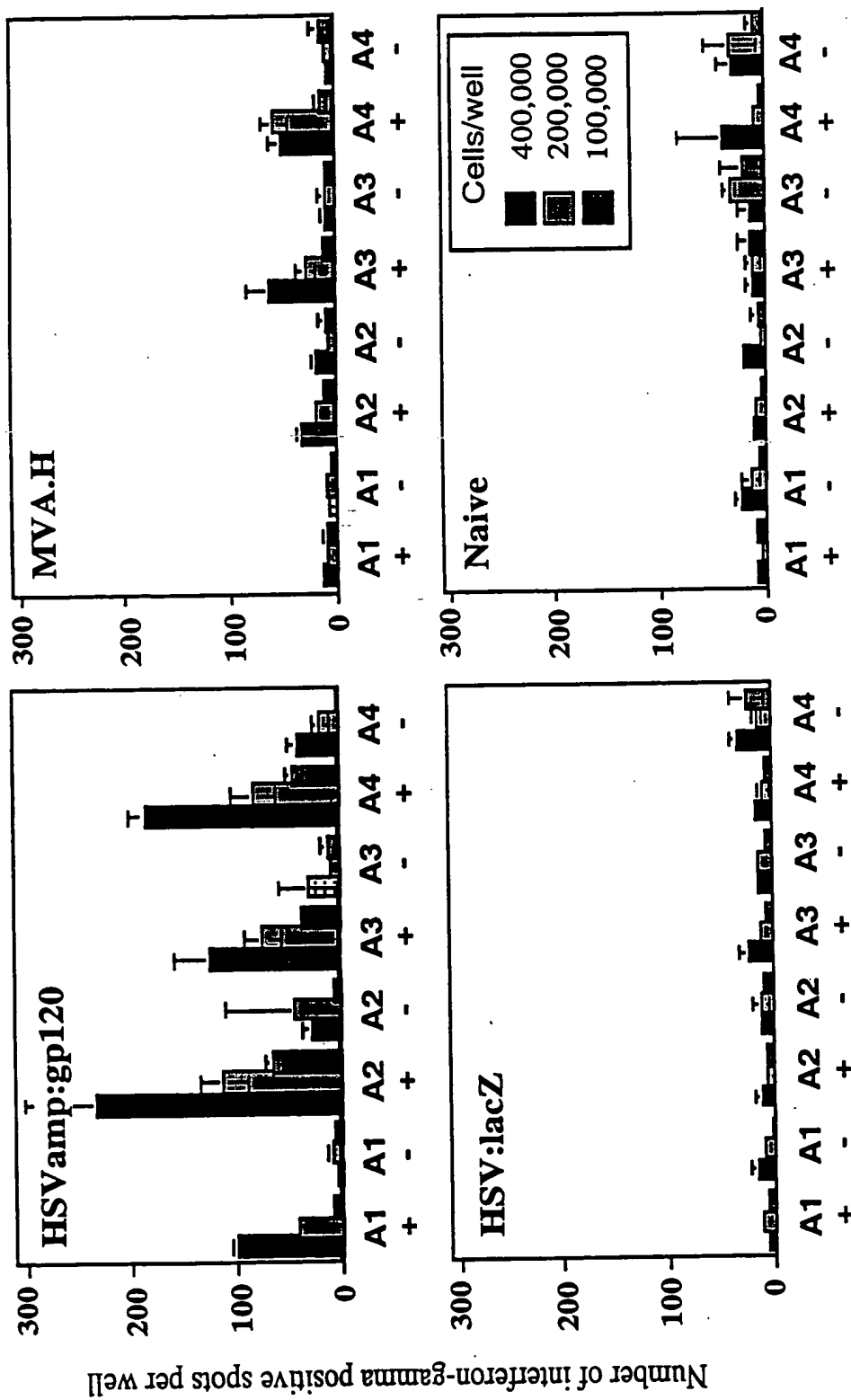
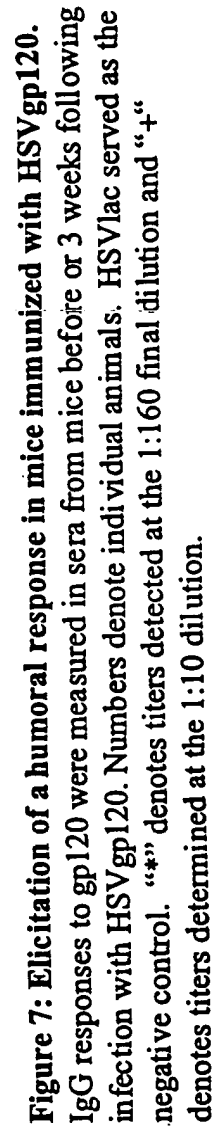
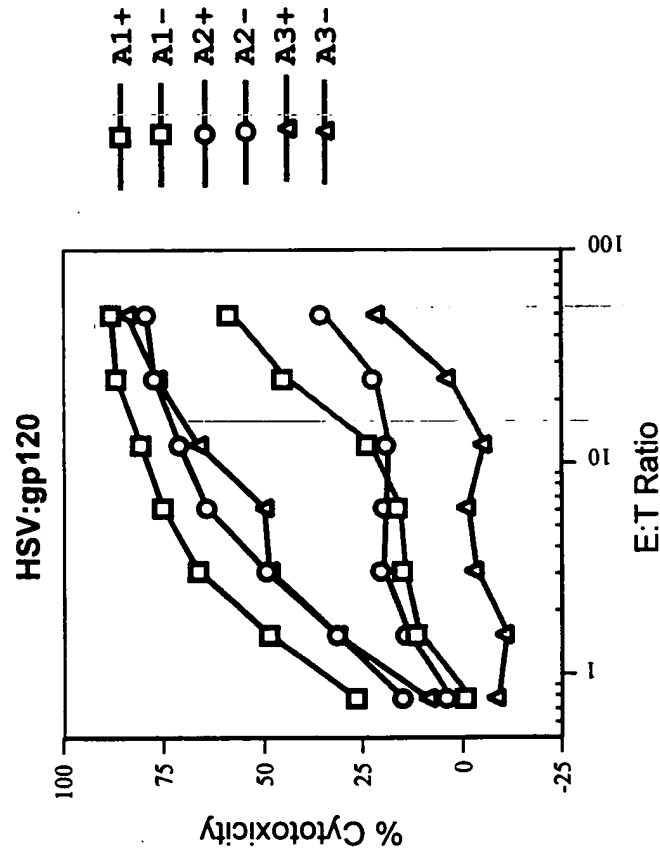


Figure 6: Immunization of mice with HSV gp120 leads to a marked cell-mediated immune response. Cellular responses to the class I-restricted peptide from gp120 (RGPGRFVTT) were measured by interferon gamma Elispot. Results from triplicate assays are shown, performed with 3 dilutions of input splenocytes. Numbers represent individual animals, with splenocytes incubated with (+) or without (-) the specific peptide. MVA.H represents a positive control (MVA encoding the V3 peptide).



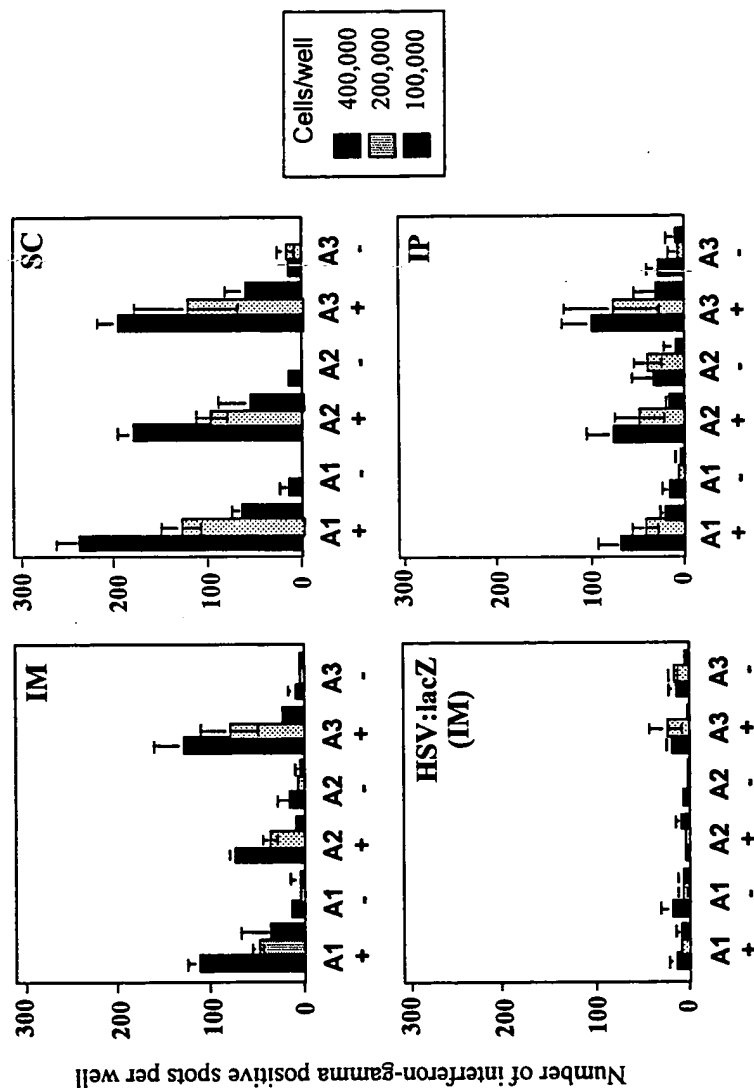
**Figure 7: Elicitation of a humoral response in mice immunized with HSVgp120.** IgG responses to gp120 were measured in sera from mice before or 3 weeks following infection with HSVgp120. Numbers denote individual animals. HSVlac served as the negative control. “\*” denotes titers detected at the 1:160 final dilution and “+” denotes titers determined at the 1:10 dilution.

### Lysis Assay (JAM)



**Figure 8. HSVgp120-mediated induction of CTL activity.** BALB/c mice were inoculated with HSV:gp120 amplicon ( $10^6$  pfu) via the intramuscular (IM; thigh) route. Animals were sacrificed 21 days later, and splenocytes harvested. Splenocytes were restimulated in the presence of LPS blasts loaded with the HIVgp120 specific peptide (RGPRAFVTI). After 5 days, these effector cells were then mixed at various ratios with radiolabeled P815 target cells, either pulsed with peptide (+; RGPRAFVTI) or unpulsed (-). Cell killing was assessed using the JAM assay method (Matzinger et al.), and data are expressed in terms of percent cytotoxicity at each effector to target (E:T) ratio. A1, 2, 3 denote data from individual animals. The data show that a single intramuscular inoculation of the HSVgp120 vector led to a strong, peptide-specific, cytotoxic effector response in these animals.





**Figure 9. Effect of route of inoculation on immune response.** BALB/c mice were inoculated with HSV:gp120 amplicon ( $10^6$  pfu) via either intramuscular (IM; thigh), subcutaneous (SC; tail base), or intraperitoneal (IP) routes; control animals received  $10^6$  pfu of the HSVlacZ vector via the IM route. Animals were sacrificed 21 days later, and splenocytes harvested. An interferon-gamma Elispot assay was then performed on these splenocytes, using either an HIVgp120 specific peptide (+; RPGRFVTT) or no peptide (-). A1, 2, 3 denote data from individual animals. See other Elispot assay for additional details. The data show that subcutaneous inoculation of the HSVgp120 vector led to the greatest level of cellular immune response in splenocytes, as defined in this assay system under the parameters used.

UPPER TABLE  
OF  
FIGURE 11

Treatment	IL-2 (pg/ml)
No virus control	461
HSVlac	N.D.
hf-HSVlac	54
HSVB7.1	173
hf-HSVB7.1	1942

Table 1: IL-2 production following transduction of CLL cells with helper virus-containing and helper virus-free amplicon stocks

N.D.=not detected

# MIDDLE TABLE

OF

FIGURE 11

Treatment	CD40L (%)	B7.1 (%)	CD40L and B7.1 (%)
HSVlac	2.0	12.5	0.5
hf-HSVlac	1.4	16.3	0.3
HSVCD40L	77.4	13.1	7
hf-HSVCD40L	48.6	41.6	14.7

Table 2: Percentage of CLL cells expressing B7.1 and CD40L following transduction with helper virus-containing and helper virus-free amplicon stocks.

LOWER TABLE  
OF  
FIGURE 11

Treatment	$\gamma$ -interferon (pg/ml)
No virus control	515
hf-HSVlac	550
hf-HSVCD40L	1088

Table 3:  $\gamma$ -interferon levels in supernatant derived from CTL assay using CLL cells transduced with helper virus-free amplicon stocks.